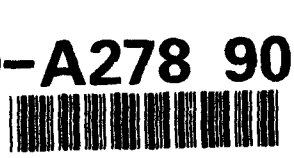

(12)

OFFICE OF NAVAL RESEARCH

FINAL REPORT

PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS/ STUDENTS REPORTS

For

Grant: N00014-88-K-0441

R&T Code 4135018

"Molecular Recognition of DNA. Synthesis of Novel Bases for Triple Helix Formation"

Peter B. Dervan

California Institute of Technology
Division of Chemistry and Chemical Engineering
Pasadena, CA 91125

1989-91

PART I

A) Completed work (1988-91)

Triple Helix Formation by Oligonucleotides on DNA Extended to the Physiological pH Range. T. J. Povsic and P. B. Dervan, ***J. Am. Chem. Soc.***, **111**, 3059 (1989).

Recognition of Thymine•Adenine Base Pairs by Guanine in a Pyrimidine Triple Helix Motif. L. C. Griffin and P. B. Dervan, ***Science***, **245**, 967 (1989).

Effects of an Abasic Site on Triple Helix Formation Characterized by Affinity Cleaving. D. A. Horne and P. B. Dervan, ***Nucleic Acids Res.***, **19**, 4963 (1991).

Recognition of All Four Base Pairs of Double-Helical DNA by Triple-Helix Formation. Design of Nonnatural Deoxyribonucleosides for Pyrimidine• Purine Base Pair Binding. L. C. Griffin, L. L. Kiessling, P. A. Beal, P. Gillespie and P. B. Dervan, ***J. Am. Chem. Soc.***, **114**, 7976 (1992).

Design of a Nonnatural Deoxyribonucleotide for Recognition of GC Base Pairs by Oligonucleotide-Directed Triple Helix Formation. J. S. Koh and P. B. Dervan, ***J. Am. Chem. Soc.***, **114**, 1470 (1992).

Flanking Sequence Effects within the Pyrimidine Triple-Helix Motif Characterized by Affinity Cleaving. L. L. Kiessling, L. C. Griffin and P. B. Dervan, ***Biochemistry***, **31**, 2829 (1992).

D) Published Chapters (1988-91)

Oligonucleotide Recognition of Double Helical DNA by Triple Helix Formation. P. B. Dervan in Oligonucleotides as Inhibitors of Gene Expression. pp. 197-210, J. S. Cohen (ed.) MacMillan Press Ltd., London (1990).

Chemical Methods for the Site-Specific Cleavage of Genomic DNA. P. B. Dervan, in Structure & Methods Vol. 1: Human Genome Initiative & DNA Recombination, pp. 37-49, R. H. Sarma and M. H. Sarma, (eds.) Adenine Press (1990).

H) Invited Presentations (1988-91)

1988

Brigham Young University: H. Smith Broadbent Lecturer
Peptide Gordon Conference: Invited Speaker
International Roche Pharma Frontiers in Chemistry Meeting, Switzerland: Plenary Lecturer
Colorado State University: Syntex Distinguished Lecturer
Universtiy of Oklahoma: Rosetta Briegel Barton Lecturer
2nd Belgian Organic Synthesis Symposium: Plenary Lecturer
Office of Naval Research: ONR Distinguished Lecturer
Eastman Kodak: Weissberger-William Lecturer
University of Kansas: Dains Lecturer
National Institutes of Health: DeWitt Stetton, Jr. Lecturer
University of Wisconsin: Perlman Lecturer
31st Annual Midwest Biochemistry Conference: Keynote Address
Columbia University: Falk-Plaut Lecturer
Albert Einstein College of Medicine: Pfizer Lecturer

1989

Frontiers in Bioorganic Chemistry, AAAS Symposium, San Francisco: Invited Lecturer
Research Perspectives in Structural Biology & Chemistry, 5th Biennial Drug Information Association Meeting, San Francisco: Invited Speaker
National Symposium on Frontiers in Science, National Academy of Sciences, Irvine: Invited Lecturer
Biotechnology and Human Genetic Predisposition to Disease, UCLA Symposia, Steamboat Springs, Colorado: Invited Lecturer
University of North Dakota: George A. Abbott Lecturer
1st Stanislao Cannizzaro Workshop, Enna, Italy: Invited Lecturer
Biomolecular Stereodynamics Conference, Albany, N.Y.: Invited Lecturer
Nucleic Acids Gordon Conference: Chairman
Heterocycles Gordon Conference: Invited Speaker
Fourth International Conference on Bioinorganic Chemistry, Cambridge, Massachusetts: Invited Lecturer
Protein Society Meeting, Seattle: Plenary Lecturer
Dedication Symposium, University of Michican: Plenary Lecturer
Pennsylvania State University: Priestly Lecturer
McGill University: Clifford B. Purves Lecturer

1990

Oxygen Radicals in Biology Gordon Conference: Invited Speaker
Frontiers in Biomedical Research, Annenberg Center: Invited Speaker
Texas A&M University: Frontiers in Chemical Research Lecturer
Boston College: University Lecturer
Symposium on Selective Transformation in Organic Chemistry, The Netherlands: Invited Speaker
University of Washington: Walker-Ames Lecturer
10th IUPAC Conference on Physical Organic Chemistry, Haifa, Israel: Plenary Lecturer
Genes as Targets for Drug Design, ACS National Medicinal Chemistry Symposium: Invited Lecturer
Symposium on the Chemical Synthesis of Antibiotics, Oiso, Japan: Plenary Lecturer
Vanderbilt University: Arthur Ingersoll Lecturer

1991

Metals in Biology Gordon Conference: Invited Speaker
35th Annual Meeting of the Biophysical Society, San Francisco: Invited Speaker
University of Montreal: BioMega Lecturer
Furman University: John Albert Southern Lecturer
Cancer Therapy into the 21st Century Symposium: Plenary Lecturer
Hoffmann La Roche: Max Hoffer Memorial Lecturer
Purdue University: H. C. Brown Lecturer
Carlton College: Frank G. and Jean M. Chesley Lecturer
University of California, Berkeley: Calvin Lecturer
Nucleic Acids Gordon Conference: Invited Speaker
32nd National Organic Symposium, Minnesota: Invited Speaker
Conference Jacques Monod "Drugs Acting on Nucleic Acids," Roscoff, France: Invited Lecturer
Stereochemistry at Sheffield, England: Invited Speaker
23rd General Assembly of Gesellshaft Deutscher Chemiker, Munich, Germany: Invited Speaker
Frontiers in Chemistry and Medicine Symposium, Glaxo UNC Conference: Invited Speaker
University of Notre Dame: Reilly Lecturer

J) Honors

1989 Alexander Todd Visiting Professor, *University of Cambridge*, England
1990 Walker-Ames Visiting Professor, *University of Washington*

K) Graduate Students and Postdoctorals Supported by ONR

Thomas J. Povsic
Linda C. Griffin
Jong S. Koh
Dr. Laura L. Kiessling
Dr. David A. Horne

L) Other Funding

Source of Support and Title	Proj Period	Direct Costs Current Year
NIH (GM-27681) Sequence-Specific Recognition of Double Helical DNA	04/01/88-03/31/91	132,998
NIH (GM-35724) Oligonucleotide-Directed Modification of DNA and RNA	01/01/90-12/31/94	169,544
NIH (GM-43966) Site Specific Chemical Methods for Cleaving Genomic DNA	07/01/89-06/30/94	159,118
***DARPA** Biopolymers: Protein:Nucleic Acid Recognition	10/01/86-09/30/91	134,177
NFCR Studies in Protein-Nucleic Acid Recognition		21,500

* Seed Grant. Renewal not planned.

PART II

A) **Principal Investigator:** Peter B. Dervan

B) **Telephone Number:** 818-356-6002

C) **Cognizant ONR Scientific Officer:** Harold Guard

D) Description of the Project

The objective of our research program is understanding the chemical principles for the sequence specific recognition of double helical DNA. The long range goal is to provide a *general solution* of uniquely recognizing any single site in the human genome (3 billion base pairs). Pyrimidine oligodeoxyribonucleotides specifically bind single sites within *large double-stranded DNA* by formation of a triple-stranded complex (Moser & Dervan, *Science* **238**, 645 (1987)). Pyrimidine oligonucleotides bind *parallel* to the purine strand in the major groove of the Watson-Crick double helical DNA *(T•AT, C+GC triplets).* Purine oligonucleotides bind *antiparallel* to the purine strand of W-C double helical DNA (G•GC and A•AT triplets) (Beal & Dervan, *Science* **251**, 1360 (1991)).

With the finding that the triple helix formation occurs at purine tracts (G,A) within large DNA, we are embarking on the construction of a *second generation* set of triple strand forming molecules for recognition of double helical DNA *at any sequence* (G, A, T, C). Novel bases will be designed that allow specific recognition of C and T bases by triple helix formation, completing the general solution to the sequence specific recognition of DNA.

E) Significant Results

Recognition of All Four Base Pairs of Double-Helical DNA by Triple-Helix Formation. Design of Nonnatural Deoxyribonucleosides for Pyrimidine•Purine Base Pair Binding. L. C. Griffin, L. L. Kiessling, P. A. Beal, P. Gillespie and P. B. Dervan, *J. Am. Chem. Soc.*, **114**, 7976 (1992).

The novel base 4-(3-benzamido)phenylimidazole was designed, synthesized and incorporated within a pyrimidine oligonucleotide and shown to recognize pyrimidine•purine base pairs over purine•pyrimidine base pairs. Such specificity allows binding by triple helix formation at an 18 base pairs site in sv 40 DNA containing *all four base pairs* at physiologically relevant conditions.

Design of a Nonnatural Deoxyribonucleotide for Recognition of GC Base Pairs by Oligonucleotide-Directed Triple Helix Formation. J. S. Koh and P. B. Dervan, *J. Am. Chem. Soc.*, **114**, 1470 (1992).

The binding of pyrimidine oligonucleotides containing cytosine at single sites on double helical DNA by triple helix formation is sensitive to pH. An

important factor is the required protonation of the cytosine N-3 in the third strand to enable the formation of two Hoogsteen hydrogen bonds (G+GC triplet). Because oligonucleotide specificity could provide a method for artificial repression of viral and pathogenic diseases, it is desirable to design and synthesize a novel base that could bind GC base pairs strongly and selectively over a wide range of intracellular pH (>7.4). The novel base, N-[1-[5-O-[bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-D-erythro-pentofuranosyl]-4,7-dihydro-3-methyl-7-oxo-1H-pyrazolo-[4,3-d]pyrimidine 5-yl]-2-methyl-propanamide (**P1**) was designed, synthesized and incorporated within a pyrimidine oligonucleotide and shown to recognize GC base pairs as selectively and strongly as C. Oligonucleotides containing P1 bases show the same specificity as C but with less pH sensitivity in triple helix formation. P1 does not require protonation in triple helix formation. Such specificity allows binding at a 15 base pairs site in plasmid DNA (pH 7.8) and a 16 base pairs site in the 3' long terminal repeat (LTR) of HIV DNA (pH 7.4).

Effects of an Abasic Site on Triple Helix Formation Characterized by Affinity Cleaving. D. A. Horne and P. B. Dervan, *Nucleic Acids Res.*, **19**, 4963 (1991).

The stability of triple helical complexes between oligodeoxyribonucleotides containing one or two abasic 1,2-dideoxy-D-ribose (Ø) residues bound to single 15-17 base pair sites within short duplex (30 mer) or plasmid DNA (4.9 kbp) was examined by affinity cleaving. The triplets Ø•AT, Ø•GC, Ø•TA and Ø•CG are significantly less stable than triplexes having the matched counterparts, T•AT, C+GC and G•TA. Generally, the decrease in binding produced by an abasic residue is at best equivalent to that observed with imperfectly matched natural base triplets with Ø•AT and Ø•GC being less stable than Ø•TA and Ø•CG triplets.

Flanking Sequence Effects within the Pyrimidine Triple-Helix Motif Characterized by Affinity Cleaving. L. L. Kiessling, L. C. Griffin and P. B. Dervan, *Biochemistry*, **31**, 2829 (1992).

Affinity cleaving studies provide evidence that nearest neighbor interactions affect the relative stabilities of triple helices. Several groups of target duplexes were synthesized with a central base triplet held constant and the adjacent 3' and 5' triplets systematically varied. By incorporating a thymidine residue with the DNA-cleaving moiety EDTA in the third strand, the relative stabilities of Hoogsteen base triplets T•AT and C+GC as well as the newly discovered base triplets G•TA, Z•TA, and D_3•CG were assessed in the context of different sequences. The T•AT triplet was shown to be relatively insensitive to substitutions in either the 3' or 5' directions, while relative stabilities of triple helices containing C+GC triplets decreased as the number of adjacent C+GC triplets increased. Triple helices incorporating a G•TA interaction were most stable when this triplet was flanked by two T•AT triplets, and were most adversely affected when a C+GC triplet was placed in the adjacent 5' direction. In contrast, complexes containing a D_3•TA interaction were destabilized when the adjacent 3' position was occupied with a C+GC triplet. The D_3•CG interaction displayed the same binding preferences. New guidelines for targeting sequences containing pyrimidine base pairs have been developed.

PART III

Research Highlight

We have cleaved a human chromosome 200 million base pairs in size *at a single site* in a background of 10 billion base pairs (*Science* 254, 1639 (1991)). We can create *in water* an ensemble of specific hydrogen bonds that can locate 50 Angstroms (Å) of DNA sequence space (15 base pairs) in 1 meter of DNA (3×10^9 base pairs). With regard to *nonenzymatic catalysis*, we now understand, in principle, how to carry out a near quantitative chemical reaction in water *at a single atom* within 10^{11} atoms of the human genome.

In unpublished work (Koshlap et al., *J. Am. Chem. Soc.*, submitted), we have demonstrated a new triple helical recognition motif. The nonnatural base D_3 binds sequence specifically by intercalation within a triple helix.

Implications

Because the DNA polymer is an *informational macromolecule* (genetic blueprint for ~100,000 gene products), this chemical approach has profound long range implications. The DNA macromolecule is a 1 meter thread that fits well within a 5 μM volume and contains 3000 volumes of information with 1000 pages/volume and 1000 letters/page. We are beginning to understand the chemical principles for locating *single sites* (word) within megabase size DNA polymers using sequence specific hydrogen bonds. The ONR supports a program to arrive at a *general* recognition code.

Within the context of molecular engineering to atomic specifications, we view nucleic acids as a 1st generation "molecular lego set" that is very stable with well-documented, reliable chemical and biological methods for synthesis and analyses.

In addition to addressing fundamental questions about molecular recognition in water (self-assembly), I believe that *key unanswered* issues in *supramolecular aqueous organic chemistry* are related to *coupling separate functions*. How do we *couple* recognition, cooperativity, allostery, metalloregulation, and atom specific chemical reactions? Nanostructures for memory storage and information transfer will become possible if a bottoms-up approach for developing the logic and tools for understanding the chemical principles underpinning this problem can be elucidated.